AMENDED CLAIMS

[received by the International Bureau on 22 July 2005 (22.07.05);
original claims 1-20 replaced by new claims 1-19 (3 pages).]

1. A method for preparing ready-to-use solid support for rapid ELISA, wherein the said method comprises addition of first monoclonal antibody, washing with buffer to remove unbound monoclonal antibody adding a stabilizer, removing excess stabilizer, air-drying of the bound stabilizer, addition of an appropriate second antibody and enzyme linked conjugate as third antibody together dissolved in buffer, lyophilising the said protein mixture and storing in a sealed package at a specified temperature.

2. A method as claimed in claim 1, wherein the first monoclonal antibody is raised against the protein/antigen to be detected.

3. A method as claimed in claim 1, wherein the first monoclonal antibody used is selected from a group consisting of monoclonal antibodies raised against Cry proteins and monoclonal antibodies against 5-enolpyruvylshikimate-3-phosphate synthase, wherein Cry protein is preferably selected from Cry1Ab, Cry1Ac Cry2Ab, Cry 9A, Cry 9B and Cry 9C.

4 A method as claimed in claim 1, wherein the buffer used for washing is phosphate buffer saline having a pH in the range of 6.8-7.2.

5. A method as claimed in claim 1, wherein buffer used for dissolving second and third antibody is selected from a group consisting of carbonate buffer and phosphate buffer, having pH in the range of 9.0-9.8.

6. A method as claimed in claim 1, wherein the stabilizer used is selected from a group consisting of Phosphate Buffered Saline, Fish Gelatin and Glycerol mixture and a Tris-buffer, Fish Gelatin and Glycerol mixture.

7. A method as claimed in claim 1, wherein the drying method used is either freeze drying or lyophilization.

8. A method as claimed in claim 1, wherein the blocking agent used is selected from the group consisting of ovalbumin, bovine serum albumin, bovine nonfat milk powder, casein, fish gelatin, porcine gelatin and lambda-carrageenan.

9. A method as claimed in claim 1, wherein the solid support used is selected from the group consisting of ELISA plate and microwell plate.

10. A method as claimed in claim 1, wherein the material for the solid support used is either polystyrene or polypropylene.

11. A method as claimed in claim 9, wherein the solid support is made of polystyrene.

12. A method as claimed in claim 1, wherein second antibody used is polyclonal antibody IgG raised against protein/antigen to be detected.

13. A method as claimed in claim 1, wherein second antibody used is polyclonal antibody IgG raised against corresponding Cry protein or IgG raised against 5-enolpyruvylshikimate-3-phosphate synthase.

14.A method as claimed in claim 1, wherein third antibody used is selected from the group consisting of polyclonal whole IgG conjugated to an enzyme, wherein whole IgG may be obtained from class Mammalia or class Aves.

15.A method as claimed in claim 14, wherein the enzyme used is selected from a group consisting of alkaline phosphatase and horseradish peroxidase

16. A rapid method for performing ELISA using ready-to-use solid support of claim 1 said method comprising steps of reconstituting the ready to use plates by adding appropriate amount of distilled water, adding test samples containing antigen/protein are dissolved in a suitable buffer, washing the plate after incubating for a required time period, followed by washing with suitable buffer, adding to the plate required chemical substrate and detecting for the presence of the antigen by measuring absorbance at a suitable wavelength.

17 A method as claimed in claim 16, wherein the chemical substrate is selected from the group consisting of para-nitrophenol phosphate, Nitro Blue Tetrazolium/5-Bromo-4-Chloro-3-Indolyl Phosphate, 2,2'-Azino-bis (3-Ethylbenz-thiazoline-6-Sulfonic Acid), o-Phenylenediamine, 3,3'-5,5'-Tetramethylbenzidine, o-Dianisidine and 5-Aminosalicylic Acid.

18. An immunoassay kit comprising of ready to use solid support of claim 1 for rapid ELISA .

19. A ready-to-use solid support of claim 1 for detection of protein or antigen

STATEMENT

The present invention provides a method for the preparation of ready to use solid support for rapid ELISA requiring much less time for preparing and reconstituting the said solid support having enhanced storage stability at both room temperature and cold conditions. This enables the method for the preparation of solid support of the present invention to be novel and inventive.

Applicant has gone through the written opinion of the International Search Authority and observe that:

1. Novelty of claims 1 to 19 has been acknowledged within the sense of Art. 33(2) of PCT.
2. Industrial applicability of claims 1 to 20 has been acknowledged.
3. In the light of Documents D_1 to D_9 inventive step of claims 1 to 20 is not established within the sense of Art.33(3) of PCT.

   In this context, Applicant believes that the ready to use solid support for rapid ELISA has been referred to only in Document D3, which is the closest prior art for the present invention. Also, Applicant wants to bring to the notice differences existing between their invention and the invention described in Document D_3 as enumerated herein below:

a). In the prior art solid support for ELISA, the primary molecules (first antibody) used is not stabilized, whereas it is stabilized in the solid support of the present invention. This difference imparts to enhanced shelf life for the ready to use solid support of present invention on storing at both room temperature and 4°C respectively.

b) Use of stabilizer in the process of present invention preserves primary molecule (first antibody) used., **whereas use of blocking agent in the prior art only blocks the unbound parts of the well present in the solid support.**

c). Reconstitution of solid support is performed using water in both present invention and Document D_3. However, the reconstitution time taken for the solid support of present invention is one sixth of the time taken for the solid support referred to in Document D_3.

d). Dying or lyophilization is not required after the addition of first antibody to the solid support in the present invention, which enables the process for the preparation of solid support to be less cumbersome and time consuming compared to the preparation of solid support of prior art.

e). Second and third antibodies are added simultaneously in the preparation of ready to use solid support of present invention which is not the case with prior art processes..The simaltaneous addition of antibodies contributes to significant reduction in the process time.

f) In the process referred to inD_3 document (Example 2, Step 4) teaches introduction of cold sample dilution medium into the frozen coated plate. It appears that the total process adapted in document D_3 is performed under cold conditions, whereas the process of present invention can be effectively even at room temperature.

g) The total time taken for the process to obtain ready to use solid support of present invention is about 24 30 hours whereas the process reported in the D_3 document to obtain ready to use ELISA plate will be around 60 hours.

4. Ref. Item VIII : Claims 19-20 do not meet the requirements of Art. 6 PCT because the subject-matter for which protection is sought is not defined at all (the solid support may be anyone used in a common immunoassay). For this reason, it is not possible to carry out a meaningful search embracing the whole scope of the claims.

Claims 19 & 20 (old numbers) have been suitably amended and re-numbered as claims 18 & 19 respectively to comply with the requirements of Art.6 PCT.

The above facts establish that ready to use solid support of the present invention is superior to the solid support reported in the prior art as far as shelf life and preparation time is concerned. This contributes to an added advantage of the present invention over the prior art invention.

The Applicant requests consideration of amended claims along with above comments.